PROCESSES AFFECTING VARIABILITY OF FLUORESCENCE SIGNALS FROM BENTHIC TARGETS IN SHALLOW WATERS

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LONG-TERM GOALS

The long term goals of this research program are to develop the biophysical tools and models that predict the sources of variability of chlorophyll fluorescence from benthic targets in shallow waters.

OBJECTIVES

The scientific objectives of the project are to relate the fluorescence lifetimes of photosynthetic targets to the amplitude-based fluorescence measurements. In so doing, laser-based fluorescence retrievals from line-scanners or other in situ instruments can be deconvoluted in the lifetime domain to reconstruct biophysical and physiological information about the photosynthetic activity of target organisms.

APPROACH

The basic approach taken is to compare high-precision, laser-induced fluorescence lifetimes of model organisms with amplitude based measurements using fast-repetition rate fluorescence techniques. The research is conducted in collaboration with Drs. Maxim Gorbunov, Zbigniew Kolber and Edward Castner. Dr. Castner is a physical chemist specializing in photochemical processes in the Department of Chemistry at Brookhaven National Laboratory. The model organisms used are primarily cultured zooxanthellae obtained from a variety of symbiotic marine invertebrates.

WORK COMPLETED

Extensive fluorescence lifetime measurements and analyses were performed to develop a data base for modeling fluorescence yields. The primary instrument used in these studies is a femptosecond Ti-sapphire laser with a photon-counting detector. The lifetime data were obtained for one emission wavelength (685 nm), and compared with simultaneous fluorescence saturation profiles obtained by fast repetition rate fluorometry.

RESULTS

A typical fluorescence decay profile is shown in Figure 1. These data reveal that when

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photosynthetic reaction centers are open (corresponding to the Fo state, i.e. in the dark), fluorescence is rapidly quenched, while upon exposure to a saturating continuous light source, or in the presence of a inhibitor of electron transport, when photosynthetic reaction centers become closed, the fluorescence lifetime increases markedly (the Fm state). The fluorescence decay profiles, such as those in Figure 1, are analyzed by a multiexponential decay function (Table 1) to obtain the component lifetimes and their amplitudes. The Fo state is dominated by a 500 picosecond lifetime, while the Fm state is reflected in a 1300 ps lifetime. The weighted averages of the component lifetimes are used to construct a single 'average' lifetime.

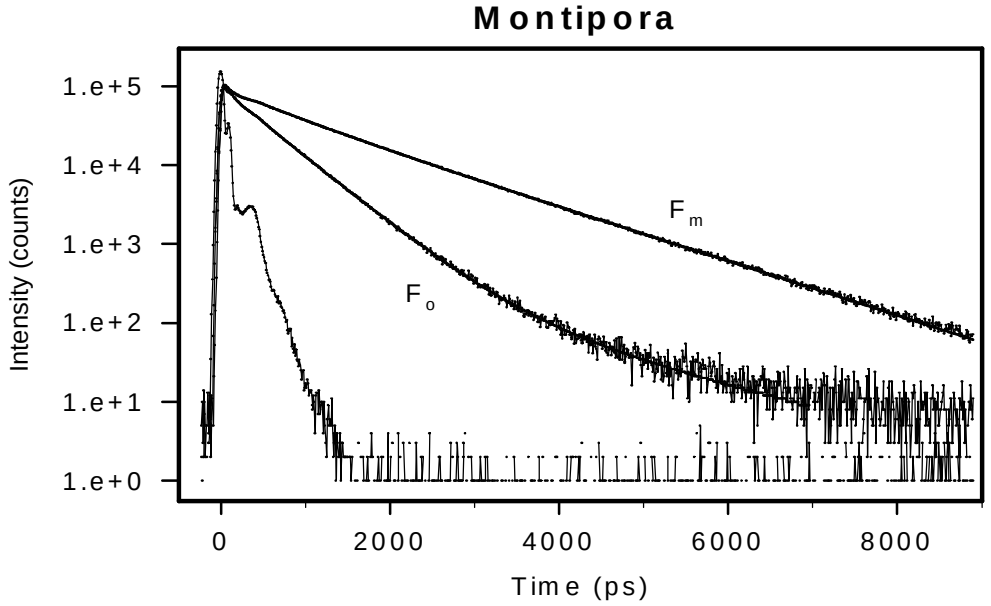

Figure 1. An example of fluorescence lifetime decays for open (Fo) and closed (Fm) photosystem II reaction centers in the zooxanthellae isolated from the fire coral, Montipora. The short lifetime curve corresponds to the laser excitation pulse.

Table 1. Lifetimes (τ_i) and relative yields (f_i) from the 4-component analysis of fluorescence decay

τ_i (ps)	f_i	τ_i (ps)	f_i
F_o		**F_m**	
25	**7.3%**	**25**	**1.3%**
167	**8.1%**	**117**	**3.2%**
504	**82.5%**	**694**	**18.3%**
1595	**2.1%**	**1285**	**77.1%**

Theoretical analysis suggests that the average fluorescence lifetime should be linearly proportional to the change in the quantum yield of fluorescence inferred from amplitude-based measurements (Falkowski and Raven 1997). Indeed, our results confirm that the average fluorescence lifetimes, obtained from five individual zooxanthellae strains, is highly correlated

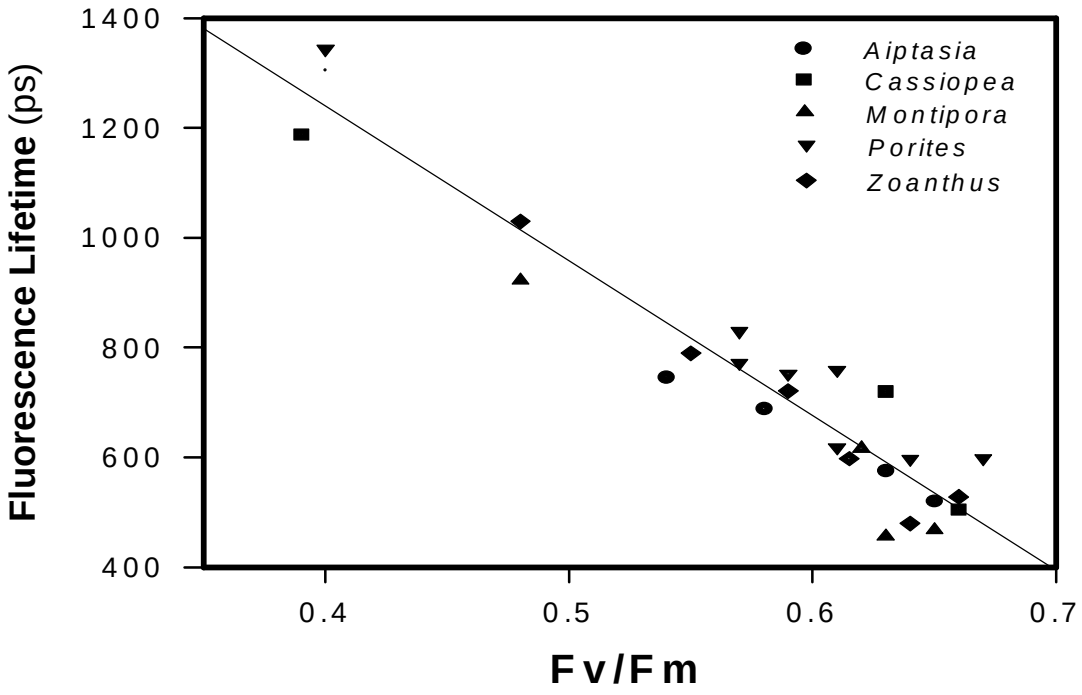

Figure 2. The correlation between the average fluorescence lifetimes, calculated from a four component exponential decay function, and the change in the quantum yield of photochemistry inferred from amplitude based measurements of variable fluorescence. The data were obtained from cultured zooxanthellae isolated from five marine invertebrates.

with the change in variable fluorescence yields (Fv/Fm) obtained with a fast repetition rate fluoreometer (Figure 2). These results strongly confirm the notion that a rapid assessment of photochemical energy conversion can be obtained non-destructively from fluorescence lifetime analyses under laboratory conditions. These data are being analyzed to infer the biophysical sources of fluorescence quenching.

IMPACT/APPLICATIONS

The results to date are the first direct comparisons of fluorescence lifetimes with amplitudes of variable fluorescence for marine unicellular algae. The potential application of the lifetime analysis to field conditions is technically challenging, but also potentially highly rewarding

from an information perspective. The potential application will be explored in the CoBOP program.

TRANSITIONS

The results presented here have not been presented publically in any forum, and hence the transition of the approach to the broader research and application community is premature.

RELATED PROJECTS

This research effort evolved from long-term support on basic understanding of fluorescence supported by NASA and DOE. The research is related to ongoing programs in both agencies.

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